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Prostate Specific or Enriched Genes as Composite Biomarkers for Prostate Cancer

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14. ABSTRACT
Purpose and scope of research: To evaluate prostate specific genes such as WDR19, NDRG1, Transgelin 2 as diagnosis and prognosis markers for prostate cancers. Major findings: (1) Serum Samples collections: We have retrieved more than 200 prostate cancer, BPH and normal matched control serum samples from the University of Washington Urology serum bank. (2) WDR19 antibody production and ELISA assay development. We have generated Rabbit monoclonal antibodies against WDR19. We have obtained six hybridoma clones. We are in the process of matching these monoclonal antibodies against each other and against two mouse monoclonal antibodies that we have previously generated to develop a sensitive ELISA assay. (3). Novel biomarker Transgelin 2. We have identified a novel biomarker Transgelin 2, which is a prostate specific gene, as a marker that showed lower expression levels in prostate cancer patients compared to normal individuals by Western Blot analysis. We are generating antibodies against Transgelin 2 and will develop an ELISA assay for Transgelin 2. (4) WDR19 as a prognosis marker for prostate cancer. We showed that WDR19 expression in prostate cancer tissues by IHC is a good prognostic marker for prostate cancer.

15. SUBJECT TERMS
WDR19, Transgelin 2, prostate cancer, biomarker, ELISA assay

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INTRODUCTION:

The long-term goal of this research is to establish a panel of multiple protein biomarkers for prostate cancer diagnosis and stratification. We have proposed to develop and optimize sandwich ELISA assays for WDR19, NDRG1, or other novel prostate specific biomarker candidates. This report will summarize the progress that we have made for our Year 2 tasks, which we laid out in the statement of work for our proposal.

BODY:

During the second year of funding period, we continued our effort developing both antibody- and mass spectrometry (MS)-based technologies for validating new prostate cancer biomarkers WDR19 and Transgelin 2 (TAGNL2).

Rabbit monoclonal antibodies were made using peptides as immunogens in the hopes that they may work better than the mouse monoclonals in producing results from a Sandwich immunoassay. This work was contracted out to Epitomics Inc. Two recombinant fragments of WDR19 were created to be used as immunogens to generate a mouse antibody (previously reported). One of these fragments contains one of the peptides used to create one of the rabbit Mabs from Epitomics Inc (batch 1). Using the mouse antibody specific for this fragment and the rabbit Mab against the peptide in the fragment together in a Sandwhich assay with the fragment as the analyte, a signal was obtained. This signal is titratable. No signal was obtained when serum samples were screened, however.

The second batch of antibodies from Epitomics have been produced and used in conjunction with the antibodies from batch 1 in sandwich immunoassays. The antibodies from batch 1 all reacted against one peptide. The antibodies from batch 2 reacted against 2 different peptides. The antibody from batch 1 and one of the batch 2 antibodies were produced from 2 different peptides which are both present in the recombinant fragment used in the aforementioned immunoassay. A sandwich immunoassay using this fragment and the two rabbit antibodies produced no signal, however promising results have been obtained when screening serum samples. 213 serum samples have been screened with the sandwich immunoassay utilizing two rabbit Mabs (detector antibody labeled with biotin). This set was composed of serum from normal controls (N= 49), patients with benign prostatic hyperplasia or BPH (N= 44), cancer patients with gleason scores <=6 or primary prostate cancer (N= 68), and cancer patients with gleason scores >=7 or advanced prostate cancer (N=52). Since there is no available standard of known concentration for this assay, a sample previously determined to give a high signal was used to build a standard curve. 56% of the serum samples produced signals above L.O.D. (2.5 x background). The sandwich immunoassay was compared against two different types of competition immunoassays. One competition assay consists of mixing a labeled antibody with analyte and then placing this mixture into a titer well with attached peptide immunogen. The peptide and analyte compete for antibody resulting in a signal that is inversely proportional to analyte concentration. The second type of competition immunoassay consists of mixing labeled peptide with analyte. This mixture is also placed into a titer well this time containing attached antibody. Again, signal is inversely proportional analyte concentration. Only 20% of the 213 samples produced signals above L.O.D in the competition assays.

A parallel comparison of immunoassay values and Western blots showed no correlation (fig. 1). This comparison was done on 35 different samples. Below is a graphical representation of the results. The western values are arbitrary. 0 = no band, 2 = weak band, and 4 = strong band.

The sandwich-immunoassay values are relative values based on a high-signal sample used as a standard.
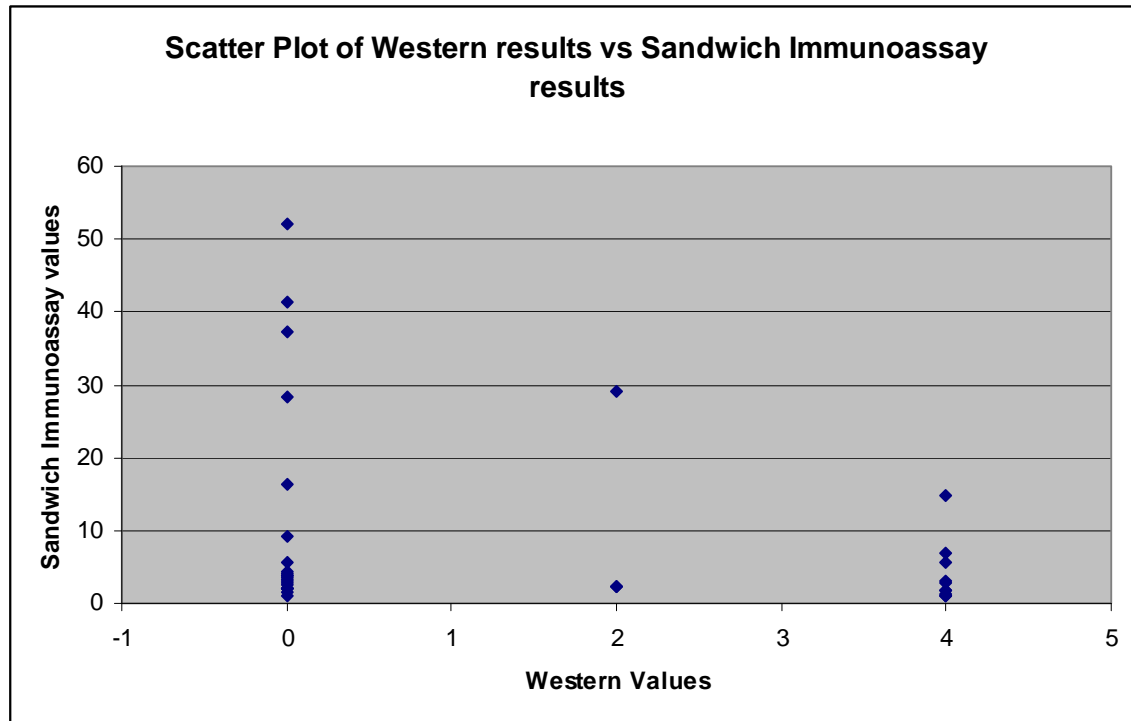
Fig.1. Cross comparison of Sandwich immunoassay and Western blots using WDR19 antibodies and prostate cancer patient sera.

Receiver-operator-characteristics analysis was performed across all sample groups using the Sandwich immunoassay values from both WDR19 and PSA. In all cases PSA outperformed WDR19 as a discriminating marker for prostate-cancer stratification. See the following plots.
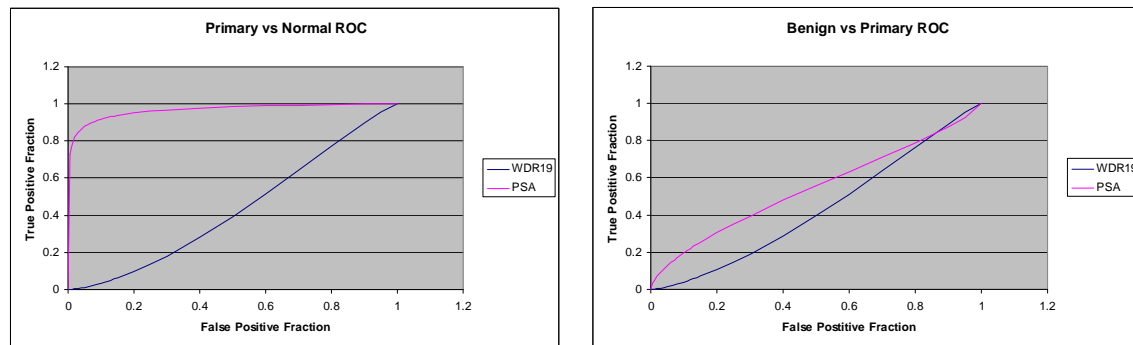

Fig.2. Receiver-operator-characteristics (ROC) analyses of WDR19 and PSA on selected prostate cancer and control serum sample groups as indicated.

PSA is very good at distinguishing between normal controls and patients with advanced prostate cancer, primary prostate cancer, and BPH (2a). PSA is weak at distinguishing between BPH and advanced prostate cancer, and between primary prostate cancer and advanced

prostate cancer.   PSA is very poor at distinguishing between BPH and primary prostate cancer (2b).

Clearly better markers than PSA are needed. Preliminary immunoassay data of WDR19 are discouraging, however more work needs to be done to clarify these results. We still have yet to determine whether the signal obtained in our immunoassays is truly due to WDR19 or to some non-specific competitor. An orthogonal assay that recapitulates or disputes the immunoassay would be ideal in getting to the bottom of this. In the works is the development of an MS-based MRM assay. For the past year, we have been developing and optimizing procedures for preparing stable isotope-labeled internal peptide standards more efficiently and cost-effectively for MRM analysis, by using an E. coli *in vitro* expression system for plasmids containing the coding sequences for concatenated peptides (QconCAT genes). Briefly, we selected tryptic peptides as standards from prostate cancer candidate proteins (e.g. WDR19, TAGNL2, and PSA) with PeptideAtlas; constructed QconCAT genes containing coding sequences for concatenated peptides; cloned genes into expression vector, performed gene transcription /translation/labeling in a cell free system; purified expressed proteins, performed trypsin digestion and clean-up to release isotopically peptide standards. We have constructed QconCAT genes for a total of 40 isotopically labeled prostate peptide standards for 6 prostate biomarker candidate genes (at least two peptides standards for each gene). The peptide standard pool includes nonglyco- and glycopeptides. Glycopeptide standards are important if protein enrichment method such as glycopeptide capture approach is needed to detect low abundance proteins in blood. All the peptide standards released from the gene products after trypsin digestion were extensively tested by LC-MS/MS on our mass spectrometer (Finnigan LCQ deca) to show that all the peptides are detectable by mass spectrometers. A selected MS spectrum was shown in figure 3.
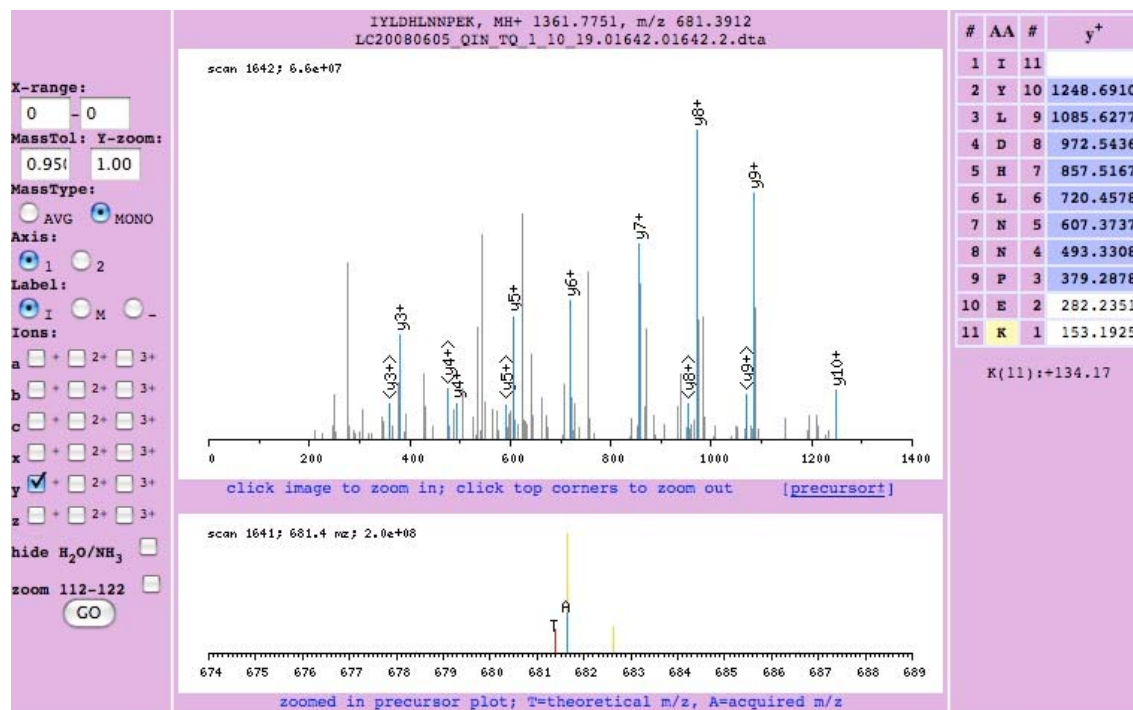
Fig 3. Heavy isotopic labeled peptide standard NSQPWHVAVYR can be successfully detected by LC-MS/MS. Mass spectrum indicates 8 product ions that are candidates for MRM transition selection.

KEY RESEARCH ACCOMPLISHMENTS:

- Developed a Sandwich immunoassay using a pair of monoclonal antibodies against WDR19, and screened more than 200 prostate cancer and control serum samples.
- Demonstrated that WDR19 is a not as good as PSA for prostate cancer diagnosis.
- Synthesized and characterized by MS 40 heavy isotopic labeled peptides from WDR19, TAGNL2, PSA, and other putative prostate cancer markers.

REPORTABLE OUTCOMES:

One manuscript submitted:

Loss of N-myc downstream-regulated gene 1 (NDRG1) increases prostate cancer cell invasion by upregulating CCL5. Lan Chun Tu, Xiaowei Yan, Biaoyang Lin, Lee Hood, Qiang Tian[1]

CONCLUSION AND FUTURE PLANS:

For ELISA assay development: A recent collaboration with ANPT Inc. (http://www.anptinc.com/), experts in immunoassay development, has been initiated in the hopes they may help us improve upon our immunoassay results. We are also in the process of developing mouse monoclonal antibodies for TAGNL2 and expect to receive them in early 2009.

For MRM assays: The peptide standards will be mixed as one pool and tested on another mass spec instrument—QTOF Premier —that is believed more similar to Waters Quattro Premier XE triple quadrupole (the mass spectrometer to be used for MRM analysis). MRM transitions (The observed m/z ratio of a peptide and its corresponding y-ion product m/z ratio) will be selected based on MS/MS data obtained and in silico means such as MRMaid, TIQAM and MRMPilot. The best two or three MRM transitions for the best peptides for each protein will be chosen for building the MRM method. The MRM method will be built for the peptide standard pool on a nanoAcquity UPLC system coupled to a Waters Quattro Premier. The theoretical retention time for each peptide will be calculated using the hydrophobicity index number of each peptide and used to calculate the expected retention times unique to this particular instrument. The results from these analyses will be converted to mzXML format and compiled; peptides initially detected will be noted and saved. All confirmed transitions will be compiled into a final method. These confirmed heavy-labeled transitions will then be combined with the non-labeled precursor and product ion transitions to create a final method in which both labeled (peptide standards) and non-labeled peptides (from control and prostate cancer serum samples) are targeted in a single analysis for prostate cancer marker discovery/validation studies.

REFERENCE:

None.

APPENDICE:

None.